International Journal of Pharmaceutics 383 (2010) 264–270

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Pharmacokinetics, tissue distribution and safety of cinnarizine delivered in lipid emulsion

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ARTICLE INFO

Article history:
Received 16 July 2009
Accepted 14 September 2009
Available online 19 September 2009

Keywords:
Cinnarizine
Lipid emulsion
Pharmacokinetics
Tissue distribution
Safety

ABSTRACT

The aim of this study was to assess the potential of cinnarizine loaded in lipid emulsion to modify the pharmacokinetics, tissue distribution and safety of cinnarizine. The cinnarizine-loaded emulsion (CLE) which can remain stable over 18-month storage at $4 \pm 2\,^{\circ}C$ was prepared by high-pressure homogenization. Nicomp™ 380 particle sizing system and HPLC were used to evaluate CLE in vitro, while UPLC/MS/MS for pharmacokinetic and tissue distribution study. The pharmacokinetics and tissue distributions of CLE were assessed by comparing with the solution form after intravenous administration to rats at a dose of 2 mg/kg. The CLE showed significant higher AUC and lower clearance and distribution volume than those of solution form. This helped cinnarizine to reach higher level in vessel, and circulate in the blood stream for a longer time resulting in better therapeutic effect. The tissue distribution exhibited significant lower uptake of CLE emulsion in lung and brain, indicating the advantage of CLE over the solution form in reducing drug precipitation in vivo and toxicity in CNS. Drug safety assessment studies including hemolysis test, intravenous stimulation and injection anaphylaxis revealed that the CLE was safe for intravenous injection.

1. Introduction

Cinnarizine (CN), a cerebral blood flow promoter (Fig. 1), is widely used for treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms (Godfraind et al., 1982; Singh, 1986) It is a weak base exhibiting pH-dependent dissolution behavior, while it dissolves readily at pH 1 (1.5 mg/mL), and it has a very low solubility at pH values greater than 4 (Ogata et al., 1986). Consequently, administration of CN as a tablets or capsule may result in a very slow bioavailability and a wide individual variation.

Intravenous CN administration is an alternative to oral administration which provides greater bioavailability, faster therapeutic effect and lower individual difference than oral dosing. However, CN with highly lipophilic and easily degradable properties made the formulation of CN quite challenging. Several intravenous dosage forms, such as water-soluble CN salt solution and CN–β-cyclodextrin inclusion complex solution have improved the solubility of CN to some extent (Wang, 2006; Zhang et al., 2004). However, these injectable dosage forms can cause pain at the injection site, venous irritation and occasionally thrombophlebitis due to acidity of vehicle and possible precipitation of the drug after intravenous administration. Attempt has been made to load the CN–cyclodextrin complexes in liposome, which could reduce the drug precipitation in vivo to some extent (Jia et al., 2007). However, none of these injectable formulations can effectively reduce the degradation of CN in solution or be subjected to thermal sterilization. This restricted their clinical applications and industrial-scale production.

The lipid emulsion having advantages in terms of high drug loading capacity, reduction in irritation or toxicity of the incorporated drug, the possibility of sustained release and industrial productivity was believed appropriate drug carrier for highly lipophilic drugs (Gettings et al., 1998; Singh and Ravin, 1986; Venkateswarlu and Patlolla, 2001). Additionally, lipid emulsion is well tolerated and exhibit lower incidence of side effects as compared to other systems on the basis of organic solvents, pH adjustment, and surface active agents, since it can separate the drug from direct contact with body fluid and tissues and reduce the opportunity of drug precipitation upon i.v. administration (Cox et al., 1991; Gen and Hunter, 1984; Wang et al., 1999). Therefore, a lipid emulsion seems to be a suitable vehicle for i.v. administration of CN.

Our previous report indicated that lipid emulsion could effectively solubilize CN. Furthermore, degradation of CN was successfully prevented by a drug loading technology which could localize CN in the interfacial lecithin layer of CN-loaded emulsion (CLE) for isolating CN from water or oil medium. The shelf life ($T_{0.9}$) of CN in emulsion estimated by degradation kinetics was 62-fold prolonged compared with solution form, demonstrating accept-

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doi:10.1016/j.ijpharm.2009.09.025

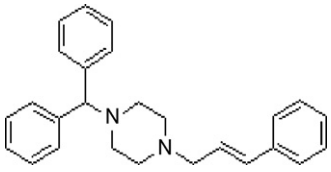

Fig. 1. Structure of cinnarizine.

able physico-chemical stability and feasibility as an intravenous lipid emulsion (Shi et al., 2009). The result of stability of CLE after 18-month storage at 4 °C was provided in our present study. The primary aim of the present study was to assess the pharmacokinetics, tissue distribution and safety of CN which could be modified by its encapsulation in the interfacial lecithin layer of lipid emulsion. CN solution was used as a control to evaluate the pharmacokinetics and tissue distribution. And the safety investigation of CLE was assessed by hemolysis test, intravenous irritation and injection anaphylaxis study to determine its function on irritation.

2. Materials and methods

2.1. Materials

The following materials were purchased from the sources in brackets: cinnarizine (Shanghai Xiandai Hashen Pharma Ltd. Co., Shangqiu, China), MCT (Lipoid KG, Ludwigshafen, Germany), egg lecithin (EPIKURON 170, PC72%, Degussa Food Ingredients, Germany), long-chain triglyceride (LCT) (Tieling Beiya Pharmaceutical Co., Tieling, China), Poloxamer 188 (Pluronic F68®) was purchased from BASF AG (Ludwigshafen, Germany), Tween80 for parenteral use (Shenyu Medicine and Chemical Industry Ltd. Co., Shanghai, China), and glycerol (Zhejiang Suichang Glycerol Plant, Zhejiang, China), diazepam (Hubei Zhongtian Aibaike Pharma Ltd. Co., Hubei, China), *N*-hexane, isopropanol (Tianjin Concord Technology Ltd., Co., Tianjin, China), sodium sulfate anhydrous (Tianjin Boya Chemical Industry Ltd., Co., Tianjin, China), egg albumen (Sigma–Aldrich Co., USA), ammonium acetate (Dima Technology Inc., Richmond Hill, USA). All chemicals and reagents used were of analytical or chromatographic grade.

All the animals used in this study were purchased from the Experimental Animal Center (Shenyang Pharmaceutical University, Shenyang, China). The experimental protocol were evaluated and approved by the University Ethics Committee for use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals.

2.2. Preparation of CLE

Firstly, 0.1% (w/v) CN together with 3% (w/v) egg lecithin was dissolved in dehydrated alcohol. After dissolving uniformly, the dehydrated alcohol was evaporated by stirring on a water bath at 80 °C until the weight of remaining lecithin–drug mixture was constant. Then, the mixture was dispersed in oil which consisted of 5% (w/v) LCT and 5% (w/v) MCT at 80 °C to obtain a clear oil phase. The aqueous phase consisting of 2.5% (w/v) glycerin, 0.03% (w/v) sodium oleate, 0.2% (w/v) Tween80, 0.2% (w/v) F68 and 0.02% (w/v) EDTA was also heated to 80 °C and agitated until uniformly dissolved. Finally, the water phase was added slowly to the oil phase with high-speed shear mixing (ULTRA RURRAX®IKA® T18 basic, Germany) at 10,000 rpm for 5 min to obtain coarse emulsion. The pH was adjusted to 8.5 with 0.1 mol/L NaOH or HCl, and the volume made up with purified water to 100 mL. After that, the coarse emulsion was subjected to high-pressure homogenization (Niro Soavi NS10012k homogenization, Via M. da Erba, 29/A-43100 Parma, Italy) at 700 bar for 8 cycles. The temperature of the entire homogenization process was maintained at 40 °C. Then, the emulsion was sealed in vials, and rotated in a 121 °C water steam bath for 15 min.

2.3. Preparation of cinnarizine solution

For the CN solution (CS), the preparation procedure was as follows. CN (100 mg), Tween80 (1 g) and 1,2 propylene glycol (10 g) were dissolved in a water solution at pH 1.0. Then, the pH was adjusted to 4.0 with 1 mol/L NaOH and the volume made up with purified water to 100 mL. The final product was subject to the sterile filtration by a 0.22 μm microporous membrane.

2.4. Characterization of CLE

The particle size and zeta potential were measured by a Nicomp™ 380 particle sizing system (Santa Barbara, USA) which was based on the principle of photon correlation spectroscopy (DLS) and electrophoretic light scattering (ELS), respectively. Samples were diluted 1:5000 with purified water adjusted to the same pH as the CLE. The entrapment efficiency (EE) of CLE was determined by measuring free CN in the aqueous phase. The CLE was subject to a Hitachi ultracentrifuge operated at 50,000 rpm for 1.5 h at 4 °C. The amount of CN in the aqueous phase was estimated by HPLC (Shi et al., 2009).

2.5. Drug analysis in vitro

A reverse phase HPLC analytical method was employed for drug analysis. A HiQ sil C18W column (250 mm × 4.6 mm, 5 μm, Kya Tech Co, Japan) was used. The mobile phase consisted of methanol–water–triethanolamine–glacial acetic acid (73:27:0.4:0.6) at a flow rate of 1.0 mL/min. The wavelength of the UV detector was set at 254 nm and the injection volume was 10 μL. The drug powder or drug-loaded emulsion was diluted with methanol to appropriate concentration before determination (Guo et al., 2006; Shi et al., 2009).

2.6. Long-term stability investigation

The stability assessment of CLE was monitored over 18 months at 4 ± 2 °C. At pre-determined time intervals, samples were removed and allowed to the room temperature. Their physical and chemical stability were evaluated by physical appearance, particle size distribution, pH value, entrapment efficiency and drug remaining.

2.7. Pharmacokinetics study

The rats weighing 200–250 g were divided into two equal groups of 6 rats per group. Groups of rats received either CLE or CS at 2 mg/kg via femoral vein. At designed times (5, 15, 30, 45 min, 1, 2, 4, 6, 8 and 12 h), the rats were anesthetized with ether and 0.3 mL blood samples were collected by retro-orbitalsinus into heparin treated tubes, and then immediately centrifuged at 4000 rpm for 15 min to obtain the plasma samples. The plasma samples were stored at −20 °C until analysis.

2.8. Tissue distribution study

The rats were randomly divided into two groups. CLE and CS were intravenously administrated via femoral vein at a dose of 2 mg/kg to rats. After injection, the rats were exsanguinated at 5, 15, 30 min, 1, 4 and 8 h after drug administration. Blood, heart, liver, spleen, lung, kidney and brain were collected. Tissue samples were

blotted with paper towel, rinsed in saline, blotted to remove excess fluid and stored at −20 °C.

2.9. Plasma and tissue sample analysis

2.9.1. Sample disposal

Twenty microliter diazepam methanol solution (40 μg/mL) as internal standard and 50 μL NaOH solution (1 mol/L) were sequentially added to 100 μL plasma or 200 μL tissue homogenate. The mixture was vortexed for 1 min in a Liquid Fast Mixer (YKH-3, Liaoxi Medical Apparatus and Instrument Factory, China), and then extracted with 3 mL *N*-hexane, isopropanol (95:5, v/v) by vortexing for 10 min. After centrifugation for 10 min at 4000 rpm, the supernatant layer was transferred to a clean tube and evaporated to dryness in a centrifugal concentrator at 40 °C (Labconco Corp., MO, USA). The residue was reconstituted in 1 mL acetonitrile–water (80:20, v/v) and a 2 μL was injected into the UPLC-ESI-MS/MS.

2.9.2. Sample determination by UPLC-ESI-MS/MS

Chromatography was performed on an ACQUITYTM UPLC system (Waters Corp., Milford, MA, USA) with a conditioned autosampler at 4 °C. The column temperature was maintained at 35 °C. The mobile phase consisting of acetonitrile and 0.05 mol/L ammonium acetate solution (80:20, v/v) was pumped through an ACQUITY UPLCTM BEH C18 column (50 mm × 2.1 mm i.d., 1.7 μm; Waters Corp., Milford, MA, USA). The injection volume was 2 μL and the partial loop mode was selected for sample injection.

The Waters ACQUITYTM TQD triple-quadrupole tandem mass spectrometer (Waters Corp., Manchester, UK) was connected to the UPLC system via an electrospray ionization (ESI) interface. The ESI source was operated in positive ionization mode with the capillary voltage set at 3.8 kV. The extractor and RF voltages were 2.0 and 0.1 V, respectively. The temperature of the source and desolvation was set at 100 and 400 °C, separately. Nitrogen was used as the desolvation gas (500 L h^{-1}) and cone gas (50 L h^{-1}). For collision-induced dissociation (CID), argon was used as the collision gas at a flow rate of 0.25 mL/min. Multiple reaction monitoring (MRM) mode was used for quantification. Transition reactions of the analytes and internal standards were given in Table 1. All data collected in centroid mode were acquired using MasslynxTM NT4.1 software (Waters Corp., Milford, MA, USA). Post-acquisition quantitative analyses were performed using a QuanLynxTM program (Waters Corp., Milford, MA, USA).

The validation of analytical method for CN in selected conditions showed that the chosen method was precise and accurate with linear response of 10–2.5 × 10^{-3} ng/mL. The lower limit of quantification (LLOQ) was 10 ng/mL. The method also showed acceptable precision and accuracy. The intra-day variation at three concentrations (20, 200, 2000 ng/mL) was 0.5–10.7% and the inter-day was less than 12.5%. These values were within the limits (<15%) specified for inter- and intra-accuracy and precision. The CN relative recovery from the plasma and tissue at three concentrations (20, 200, 2000 ng/mL) was 92.8–100.2% and 84.3–108.2%, respectively.

2.9.3. Pharmacokinetics and statistical analysis

The data were analyzed by drug and statistics (DAS) version 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China). The pharmacokinetic and tissue distribution results were analyzed statistically using the Student's independent sample *t*-test and expressed as one-way *p* value. When comparisons between groups yielded a value for $p < 0.05$, the difference between the groups was considered statistically significant. The statistical analyses were preformed by the statistical package for social science (SPSS, version 11.5).

Table 1
Transition reactions of the analyte and internal standard.

Molecule	Transition	Dwell (s)	Cone voltage (V)	Collision energy (eV)
Cinnarizine	369.2 → 166.9	0.1	30	15
Diazepam	285.2 → 193.1	0.1	50	30

2.10. Intravenous injection safety assessment

2.10.1. Hemolysis test

Rabbit blood was applied to test the hemolysis effect of CLE. Briefly, 10 mL of rabbit blood was obtained from arteria cruralis and the fibrinogen was removed by stirring with glass rod. Ten milliliter of 0.9% saline injection solution was added into defibrinogen blood sample, and supernatant was removed after centrifugation at 1500 rpm for 15 min. The erythrocytes at the bottom of centrifuge were washed three times (centrifugation followed by re-dispersion) with 0.9% saline injection solution. Finally, 2% erythrocyte standard dispersion was obtained by adding adequate amount of saline injection to the tube.

Different amounts of CLE (0.993 mg/mL) volume of 0.1, 0.2, 0.3, 0.4 and 0.5 mL were added into five tubes with 2.5 mL of 2% erythrocyte dispersion in each. Then, appropriate amount of 0.9% saline injection was added in every tube to get a final volume of 5 mL. Negative control was a drug free solution which contained 2.5 mL 2% erythrocyte dispersion and 2.5 mL 0.9% saline injection. While the positive control was prepared by addition of 2.5 mL water into 2.5 mL 2% erythrocyte. After vortex, the tubes were incubated at 37 °C and observed microscopically from 15 min to 4 h.

2.10.2. Intravenous irritation assessment

Three rabbits weighting 2.0–3.0 kg were used for this study. Each rabbit was injected with a daily dose of 0.93 mg/kg of CLE into the right ear-border vein for 3 days. An equivalent volume of 0.9% saline injection was given into the left ear-border vein as control. After injection, paradoxical reaction at the injection site was recorded. The rabbits were sacrificed 48 h after the last administration, and the ears were cut and fixed in 10% liquor formaldehyde. At the localizations of 1 and 5 cm from the injection site to proximal part, histological sections were prepared for histopathological examination.

2.10.3. Injection anaphylaxis

Guinea pigs (220–270 g) were randomly assigned to four groups ($n = 6$): (1) negative control group (0.9% saline injection); (2) positive control group (0.3% egg albumen solution); (3) CLE (3.1 mg/kg) and (4) CLE (6.2 mg/kg). Animals were intraperitoneally injected every other day for three times, and the administration volumes of negative and positive groups were consistent with Group (4). Ten days after the last administration, animals in each group were injected with a challenge dose of corresponding solution into the vein at the lateral of crus curvilineum (challenge dose was 2-fold of the administration dosage). The anaphylactic response was recorded in 3 h after the challenge injection.

3. Results and discussion

3.1. Characterization of CLE

The mean diameter of CLE given by In-Wt Gaussian distribution was 131.6 ± 48.40 nm. Besides, Gaussian distribution of CLE also provided the cumulated results of particles. It was shown in Fig. 2 that all the particles were smaller than 597 nm, in which 90% were less than 197.0 nm. The zeta potential of CLE was −28.45 mV and the pH value of CLE was determined as 8.35. The amount of CN

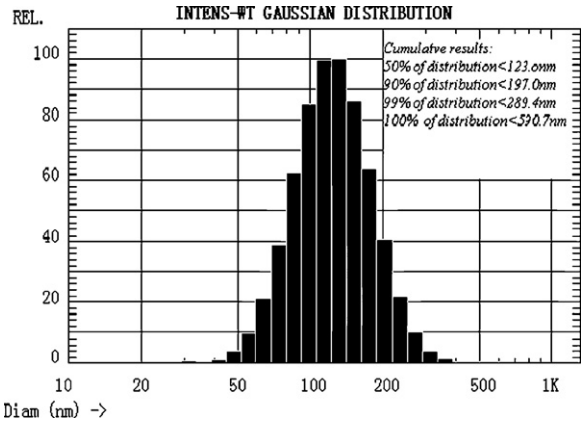

Fig. 2. In-Wt Gaussian distribution of CLE.

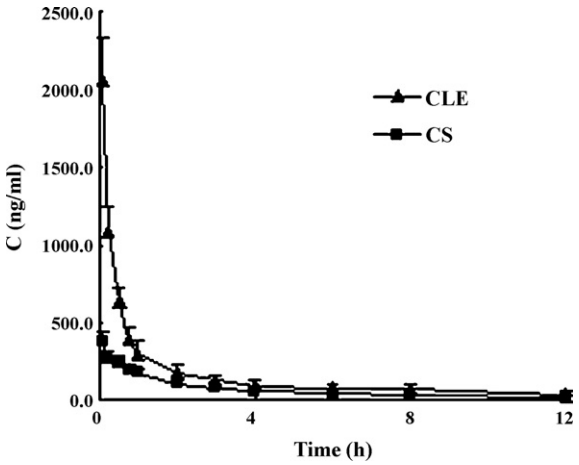

Fig. 3. Mean plasma concentration–time curves of CN after intravenous CLE and CS administration of 2 mg/kg to rats.

incorporated in emulsion was 97.3% and the formulation contained 0.993 mg/mL CN.

3.2. Long-term stability of CLE

The long-term stability investigation has been carried out for 18 months. The results from Table 2 showed that the parameters which were applied to evaluate the physico-chemical stability of CLE did not significantly change during 18-month storage at $4 \pm 2\,^{\circ}C$, indicating excellent physical and chemical stability. Hence, it can be concluded that CLE is an excellent intravenous administration system, which can undergo the real storage conditions for 18 months.

3.3. Pharmacokinetic study

The pharmacokinetic investigation was carried out to compare the CLE and CS by determines the residual concentration of CN in rat plasma up to 12 h after i.v administration. After i.v. administration of CN at dose of 2 mg/kg, no obvious side effects were observed from the rats in the CLE group. However, three rats in the CS group occurred slightly convulsion which was disappeared in 1.5 min. The mean plasma concentration–time profiles for CLE and CS injection were shown in Fig. 3 fitting a three-compartment model with a weighting factor of $1/cm^3$. The concentrations of CN in the plasma declined biexponentially and were higher for CLE than CS at all time points, especially at the initial time points (Fig. 3). The main pharmacokinetic parameters were shown in Table 3. It was exhibited that the CLE had a 2-fold increase in AUC when compared to the solution. Moreover, apparent volume of distribution (V_{ss}) and clearance (CL) after administration of CLE were 2.04 and 2.17 times lower than those of CS, respectively. On the other hand, the half-life of CN did not differ significantly between the two formulations.

The plasma pharmacokinetics of CN after i.v. administration of CLE and CS followed a three-compartment model which presented a very fast distribution phase followed by one slower and another very slow elimination phase. The significantly increased AUC and decreased CL and V_{ss} of CLE are likely due to prevention of precipitation that may have been influenced by drug carrier. CN as a weak base drug exhibits pH-dependent dissolution behavior, while it dissolves readily at pH 1 (1.5 mg/mL), and it has a very low solubility at pH values greater than 4 (Ogata et al., 1986). The current solution formulation accommodated CN by adjusting pH acid. Consequently, CN may precipitate readily at physiological pH after i.v. administration of CS. The precipitated CN will be rapidly distributed and penetrated into some tissues. However, when the drug is loaded in a lipid emulsion vehicle, it needs more time to be release from the CLE into blood. Since the drug is embedded in the lipophilic group of the phospholipids molecules to form a tight combination with phospholipids, resulting in its localization in the interfacial lecithin layer of CLE (Shi et al., 2009). This structure can more effectively delay the penetration of free CN into tissues, altering the distribution of CN. Therefore, it can be concluded that the change of CN in the pharmacokinetics characterization, imposed by its encapsulation in the interfacial layer of CLE, has led to an increase of AUC as a result of a reduction in CL and V_{ss}. However, this behavior has not significantly affected the metabolism rate to CN in rats, since the mean residence time of CLE did not change obviously compared with that of CS.

The improved pharmacokinetics behavior of CLE may be attributed to higher AUC and slower clearance of CLE than those of CS, which enable CN to reach higher level in vessel, and circulate in the blood stream for a longer time. This behavior can help the drug more effectively act on the receptors of vascular contraction, thereby reducing the availability of free calcium ions and augmenting red blood cell deformability and anti-vasoconstrictive effects

Table 2
Characterizations of CLE during 18 months investigation at $4 \pm 2\,^{\circ}C$.

Characterization of CLE	0 time	Storage at $4 \pm 2\,^{\circ}C$ for 18 months
Physical appearance	Good	Good
pH value	8.49	8.17
Particle size distribution (nm)	158.9 ± 48.56	163.3 ± 56.03
Entrapment efficiency	99.2	97.2
Zeta potential	−26.34	−24.35
Drug remaining	100.1	98.9

Table 3
Pharmacokinetic parameters after i.v. administration of CLE and CS at a dose of 2 mg/kg.

Parameter	Unit	CLE	CS
AUC[a]	$\mu g/L\,h^{-1}$	1879.556 ± 395.048	865.725 ± 203.626
AUMC[a]		4843.436 ± 1886.086	2927.055 ± 1012.535
MRT	h	5.814 ± 4.870	5.840 ± 2.085
$t_{1/2}$	h	5.177 ± 1.022	4.953 ± 1.991
CL[a]	L/h/kg	0.947 ± 0.249	2.055 ± 0.576
V_{ss}[a]	L/kg	6.871 ± 1.432	14.018 ± 5.598

The data are mean ± S.D. ($n = 6$). $t_{1/2}$, half-life; AUC, area under the concentration–time curve; AUMC, area under the cross-product of the time and plasma concentration–time curve; MRT, mean residence time; CL, clearance; V_{ss}, steady-state apparent volume of distribution.

[a] $p < 0.05$.

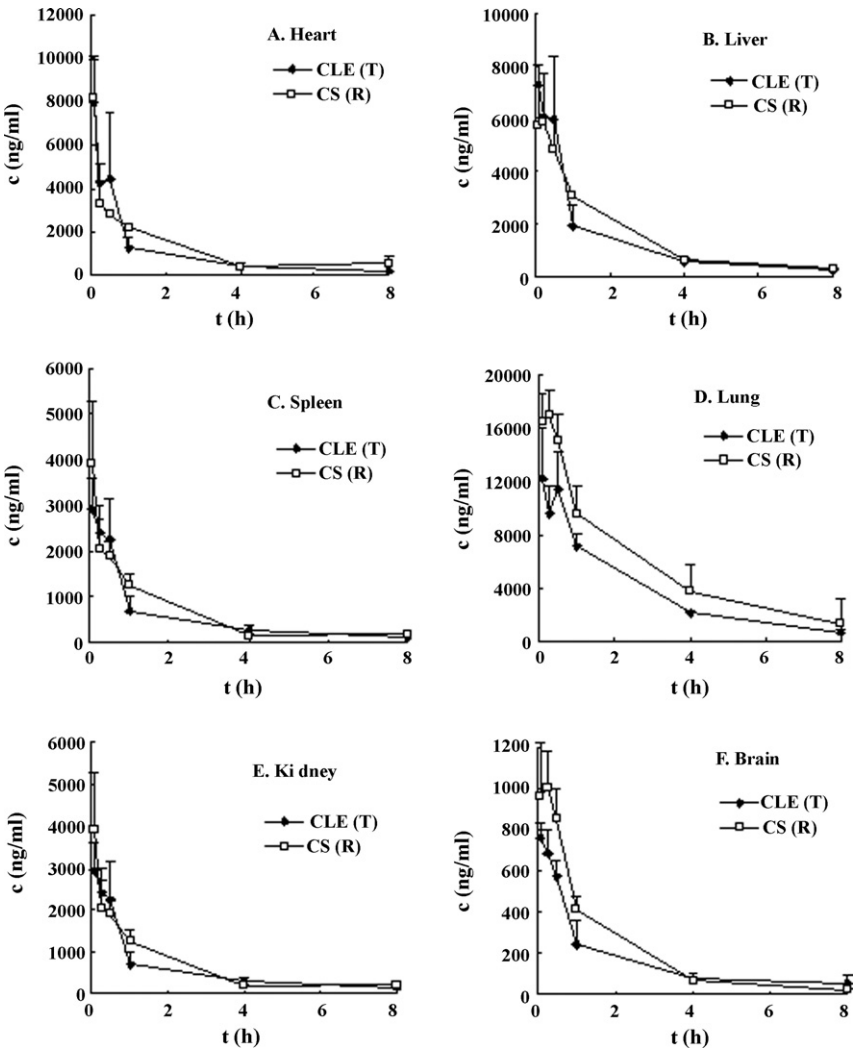

Fig. 4. Tissue distribution curves of CN after intravenous CLE and CS administration of 2 mg/kg.

(De Cree et al., 1979; Schuermans et al., 1971; Verhaegen et al., 1974).

3.4. Tissue distribution study

Tissue concentrations of CN after i.v. administration of CLE and CS were shown in Fig. 4, and AUCs in different tissues reported in Table 4. The CN concentration after 5 min of i.v. administration of CLE was highest in lung, followed by heart, liver, spleen, kidney and brain (Fig. 4). As shown in Table 4, significant lower AUCs ($p < 0.05$) of CLE were detected in lung and brain compared with those of CS.

Table 4
Comparison of tissue AUCs of CN after i.v. administration of CLE and CS at a dose of 2 mg/kg.

Tissues	AUC (mg/L h^{-1})	
	CLE	CS
Heart	7.839 ± 0.459	9.235 ± 0.998
Liver	10.344 ± 0.326	11.957 ± 0.626
Spleen	7.682 ± 1.117	9.444 ± 1.815
Lung[a]	29.544 ± 1.471	44.579 ± 16.306
Kidney	4.293 ± 0.505	4.941 ± 0.481
Brain[a]	1.296 ± 0.120	1.680 ± 0.165

Data are shown as mean ± S.D., $n = 3$.
[a] Statistically significant when AUC of CLE compared with CS in corresponding tissue at $p < 0.05$.

When lipid emulsions were administrated by i.v. injection, they are usually taken up by the (reticuloendothelial system) RES in liver, spleen and lung (Yamaguchi et al., 1984; Mizushima et al., 1982). The mechanism of uptake the lipid emulsions has been demonstrated to be either by phagocytosis or endocytosis (Yanagikawa, 1982). Unlike lipid emulsions described as above, the AUCs of CLE in liver and spleen were not increased in comparison of CS. Furthermore, the AUC of CLE in lung was significantly lower than that of CS by approximately 2-fold. This phenomenon can be attributed to the size and composition of CLE. Since small emulsions (<0.2 μm) could avoid the trapping by RES (Liang, 2005; Takino et al., 1994). In addition, the composition of the phospholipids used in emulsion could influence the ability to be metabolized by lipoprotein lipase resulting in low RES uptake (Kawakami et al., 2000). On the other hand, when CS was administrated by i.v. injection, different size of CN crystals might precipitate in vessel at physiological pH, affecting the target of CN. The smaller precipitation of CN (0.2–7 μm) may be also recognized and trapped by macrophages into the RES of liver, spleen and lung. But the larger precipitations (>7 μm) will be preferentially trapped in the capillary bed of the lung (Abra et al., 1984; Liang, 2005). So, it is reasonable that the AUC of CS in lung was significantly higher than that of CLE. However, because the lung was the favourite site of accumulation of CN (Table 4), a huge amount

of large particles transiently accumulated in the lung would induce the symptom of pulmonary embolism, which can explain the phenomenon why convulsion was seen in rats immediately after i.v. administration of CS and disappeared in 1.5 min. Therefore, it can be concluded that the CLE was safer on alleviating the side effect of pulmonary embolism to some extent.

As Fig. 4(F) shown, some CN could cross the blood-brain barrier (BBB) and distributed in the brain. Table 4 showed that the AUC of CN in brain was significantly lower in CLE than CS-treated rat ($p < 0.05$). The amount of the drug across the BBB is dependent on the lipophilicity and molecular weight (Kreuter, 2001). So, it is reasonable that free CN which owns small molecule weight and highly lipophilic property can easily transport across brain endothelial cells by passive diffusion. Whereas, when CN was incorporated in the lipid emulsion, the vehicle might restrict the release of the drug, thereby reducing its opportunity of crossing the BBB. Another reason of the lower uptake of CLE in brain was ought to be the electrical repulsive force between the surface of CLE (−28.45 mV) and brain endothelial cells which could restrict the movement of CLE across the BBB. However, the higher concentration of free CN across the BBB might be the possible reason behind the toxicity of the drug during the therapy. It was reported that the delivery of CN across BBB could act on the Central Nervous System (CNS) inducing neurologic adverse effect, for instance, drowsiness, asthenia, headache, tremor, hypokinesia, Parkinsonism and depression (Castaneda-Hernandez et al., 1993; Negrotti et al., 1992). But interestingly, the significantly lower concentration of CN across the BBB indicated the potential advantage of the lipid emulsion system over the free drug formulation on reducing the toxicity in CNS.

3.5. Intravenous safety assessment

3.5.1. Hemolysis test

Complete hemolysis was observed in tube of positive control at 15 min presenting as the red clear-diaphanous, and no erythrocyte survived at the bottom of the tube. While the erythrocyte precipitated at the bottom of other six tubes (negative control and tested drug solution with different concentration of CN) and dispersed after shaking in the 4 h observation. This phenomenon demonstrated that the CLE at concentration of 0.993 mg/mL would not induce hemolysis or erythrocyte agglutination at 37 °C.

3.5.2. Intravenous irritation assessment

After a 3-day administration of CLE and 0.9% saline injection, no erythema, edema and tissue necrosis was observed at the injection site. The histopathologic examination of the rabbit ear-border vein indicated that no thrombus, swelling or hyperplasia of endothelial cells was appeared in the blood vessel. In addition, there were not pathological changes such as hemorrhage, edema, necrosis and inflammatory cell infiltrate in the vessel wall and surrounding tissues. The histopathologic examination results of the rabbit ears administrated with CLE were similar to those of the control group. All the results above indicated that no intravenous irritation was found in the ear vein of rabbit after i.v. administration of CLE at a dosage of 0.93 mg/kg.

3.5.3. Injection anaphylaxis

All the groups of the guinea pigs were eucrasia during and after the administration. After challenge, in positive control, significant anaphylaxis symptom such as dyspnea and convulsion was observed and all the guinea pigs were died within 2 min. However, the CLE treated group and negative control group did not respond to the last challenge. Therefore, it can be concluded that CLE used intravenously at dosage of 6.2 mg/kg did not cause hypersensitivity.

4. Conclusion

In conclusion, the lipid emulsion delivery system has been found to be a suitable for CN delivery. Except for the excellent physicochemical stability, CLE may offer improved pharmacokinetics and tissue distribution by increasing the bioavailability and reducing side effects such as drug precipitation in vivo, pulmonary embolism and toxicity in CNS. In addition, the intravenous safety investigation proved that CLE was safe as an intravenous injection. Therefore, CLE demonstrated great potential for clinical applications and could be produced on an industrial scale.

Acknowledgements

Professor Yingliang Wu from Department of Pharmacology, Shenyang Pharmaceutical University is kindly acknowledged for their assistance in the safety testing. Dr. David B. Jack is gratefully thanked for correcting the manuscript.

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